

## REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

The use of triple resonance (TR) nuclear magnetic resonance (NMR) experiments for the resonance assignment of polypeptide chains *via* heteronuclear scalar connectivities is a standard approach which neatly complements the assignment protocol based on  $^1\text{H}$ - $^1\text{H}$  nuclear Overhauser effects (NOE). In addition, triple resonance NMR spectra are highly amenable to a fast automated analysis, yielding the  $^{13}\text{C}^{\alpha/\beta}$  chemical shifts at an early stage of the assignment procedure. This enables both, the identification of regular secondary structure elements without reference to NOEs and the derivation of  $(\phi,\psi)$ -angle constraints which serve to reduce the number of cycles consisting of nuclear Overhauser enhancement spectroscopy (NOESY) peak assignment and structure calculation.

NMR assignments are prerequisite for NMR-based structural biology and, thus, for high-throughput (HTP) structure determination in structural genomics and for exploring structure-activity relationships (SAR) by NMR for drug discovery. The aims of structural genomics are to (i) explore the naturally occurring “protein fold space” and (ii) contribute to the characterization of function through the assignment of atomic resolution three-dimensional (3D) structures to proteins. It is now generally acknowledged that NMR will play an important role in structural genomics. The resulting demand for HTP structure determination requires fast and automated NMR data collection and analysis protocols.

The establishment of a HTP NMR structural genomics pipeline requires two key objectives in data collection. Firstly, the measurement time should be minimized in order to (i) lower the cost per structure and (ii) relax the constraint that NMR samples need to be stable over a long period of measurement time. The recent introduction of commercial cryogenic probes promises to reduce measurement times by about a factor of ten or more, and will greatly impact the realization of this first objective. Secondly, reliable automated spectral analysis requires recording of a “redundant” set of multidimensional NMR experiments each affording good resolution (which requires appropriately long maximal evolution times in all indirect dimensions). Concomitantly, it is desirable to keep the total number of NMR spectra small in order to minimize “interspectral” variations of chemical shift measurements, which may impede automated spectral analysis. Straightforward

consideration of this second objective would suggest increasing the dimensionality of the spectra, preferably by implementing a suite of four- or even higher-dimensional NMR experiments. Importantly, however, the joint realization of the first and second objectives is tightly limited by the rather large lower bounds of higher-dimensional TR NMR measurement times if appropriately long maximal evolution times are chosen.

Hence, “sampling limited” and “sensitivity limited” data collection regimes are distinguished, depending on whether the sampling of the indirect dimensions or the sensitivity of the multidimensional NMR experiments “per se” determines the minimally achievable measurement time. As a matter of fact, the ever increasing performance of NMR spectrometers will soon lead to the situation where, for many protein samples, the sensitivity of the NMR spectrometers do not constitute the prime bottleneck determining minimal measurement times. Instead, the minimal measurement times encountered for recording conventional higher-dimensional NMR schemes will be “sampling limited,” particularly as high sensitivity cryoprobes become generally available. As structure determinations of proteins rely on nearly complete assignment of chemical shifts, which are obtained using multidimensional  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^1\text{H}$ - TR NMR experiments, the development of TR NMR techniques that avoid the sampling limited regime represents a key challenge for future biomolecular NMR methods development.

Reduced dimensionality (RD) TR NMR experiments, designed for simultaneous frequency labeling of two spin types in a single indirect dimension, offer a viable strategy to circumvent recording NMR spectra in a sampling limited fashion. RD NMR is based on a projection technique for reducing the spectral dimensionality of TR experiments: the chemical shifts of the projected dimension give rise to a cosine-modulation of the transfer amplitude, yielding peak doublets encoding  $n$  chemical shifts in a  $n-1$  dimensional spectrum. As a key result, this allows recording projected four-dimensional (4D) NMR experiments with maximal evolution times typically achieved in the corresponding conventional 3D NMR experiments. Furthermore, axial coherences, arising from either incomplete insensitive nuclei enhanced by polarization transfer (INEPT) or heteronuclear magnetization, can be observed as peaks located at the center of the doublets. This allows both the unambiguous assignment of multiple doublets with degenerate chemical shifts in the other dimensions and the identification of cross peak pairs by symmetrization of spectral strips about the position of the central peak. Hence, observation of central peaks not

only restores the dispersion of the parent, higher-dimensional experiment, but also provides access to reservoir of axial peak magnetization. Historically, RD NMR experiments were first designed to simultaneously recruit both  $^1\text{H}$  and heteronuclear magnetization for signal detection, a feature that has also gained interest for improving transverse relaxation-optimized spectroscopy (TROSY) pulse schemes. Moreover, RD two-spin coherence NMR spectroscopy, subsequently also called zero-quantum/double-quantum (ZQ/DQ) NMR spectroscopy, served as a valuable radio-frequency (r.f.) pulse module for measurement of scalar coupling constants and cross-correlated heteronuclear relaxation.

The present invention is directed to overcoming the deficiencies in the art.

The rejection of claims 91-132 under 35 U.S.C. § 112 (1<sup>st</sup> para.) for lack of enablement is respectfully traversed in view of the above amendments.

The rejection of claims 91-102, 131, and 132 under 35 U.S.C. § 103(a) for obviousness over Szyperski et al., “Sequential Resonance Assignment of Medium-Sized  $^{15}\text{N}/^{13}\text{C}$  -Labeled Proteins with Projected 4D Triple Resonance NMR Experiments,” *J. Biomol. NMR* 11:387-405 (1998) (“Szyperski”) in view of Fernández et al., “NMR With  $^{13}\text{C}$ ,  $^{15}\text{N}$  -Doubly-Labeled DNA: The *Antennapedia* Homeodomain Complex With a 14-mer DNA Duplex,” *J. Biomol. NMR* 12:25-37 (1998) (“Fernández”) or Gehring et al., “H(C)CH-COSY and (H)CCH-COSY Experiments for  $^{13}\text{C}$ -Labeled Proteins in  $\text{H}_2\text{O}$  Solution,” *J. Magn. Reson.* 135(1):185-193 (1998) (“Gehring”) and Yamazaki et al., “Two-Dimensional NMR Experiments for Correlating  $^{13}\text{C}\beta$  and  $^1\text{H}$   $\delta/\epsilon$  Chemical Shifts of Aromatic Residues in  $^{13}\text{C}$ -Labeled Proteins Via Scalar Couplings,” *J. Am. Chem. Soc.*, 115:11054-11055 (1993) (“Yamazaki”) is respectfully traversed.

Szyperski describes the use of projected four-dimensional (4D) triple resonance NMR experiments for the efficient sequential resonance assignment of  $^{15}\text{N}/^{13}\text{C}$ -labeled proteins, where a RD three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}\underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment is recorded either in conjunction with a RD 3D  $\text{HNN}<\!\!\underline{\text{CO,CA}}\!\!>$  NMR experiment or with a RD 3D HNNCAHA NMR experiment. However, Szyperski does not teach or suggest conducting a RD 3D  $\text{H,C,C,H-COSY}$  NMR experiment or a RD two-dimensional (2D)  $\text{HB,CB,(CG,CD),HD}$  NMR experiment, as claimed.

Fernández teaches obtaining  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{15}\text{N}$  NMR assignments for a doubly-labeled 14-base pair DNA duplex in solution, both in the free state and complexed with the uniformly  $^{15}\text{N}$ -labeled *Antennapedia* homeodomain. The resonance assignments are obtained

in three steps: (i) identification of the deoxyribose spin systems via scalar couplings using 2D and 3D HCCH-COSY and soft-relayed HCCH-COSY; (ii) sequential assignment of the nucleotides via  $^1\text{H}$ - $^1\text{H}$  nuclear Overhauser effects (NOEs) observed in 3D  $^{13}\text{C}$ -resolved NOESY; and (iii) assignment of the immino and amino groups via  $^1\text{H}$ - $^1\text{H}$  NOEs and  $^{15}\text{N}$ - $^1\text{H}$  correlation spectroscopy. However, Fernández merely discloses conventional HCCH-COSY NMR experiments and does not in any way teach or suggest conducting any reduced dimensionality (RD) NMR experiments, let alone a RD 3D H,C,C,H-COSY NMR experiment. Thus, Fernández cannot overcome the above-noted deficiencies of Szyperski.

Gehring discloses three experiments for identifying carbon and proton sidechain resonances in  $^{13}\text{C}$ -labeled proteins. The first experiment is an improved H(C)CH-COSY experiment comprising the application of gradients for coherence selection and a reduction in the phase cycle. The second experiment is a new (H)CCH-COSY experiment with two carbon dimensions. The third experiment is a 2D proton-edited (H)C(C)H-COSY experiment that allows suppression of methylene resonances. However, Gehring merely discloses conventional HCCH-COSY NMR experiments and does not in any way teach or suggest conducting any reduced dimensionality (RD) NMR experiments, let alone a RD 3D H,C,C,H-COSY NMR experiment. Thus, Gehring cannot overcome the above-noted deficiencies of Szyperski.

Yamazaki discloses two-dimensional NMR experiments,  $(\text{H}\beta)\text{C}\beta(\text{C}\gamma\text{C}\delta)\text{H}\delta$  and  $(\text{H}\beta)\text{C}\beta(\text{C}\gamma\text{C}\delta\text{C}\varepsilon)\text{H}\varepsilon$ , for correlating  $^{13}\text{C}\beta$  and  $^1\text{H}$   $\delta/\varepsilon$  chemical shifts of aromatic residues in  $^{13}\text{C}$ -labeled proteins based on scalar connectivities. However, Yamazaki merely discloses a conventional 2D  $(\text{H}\beta)\text{C}\beta(\text{C}\gamma\text{C}\delta)\text{H}\delta$  NMR experiment and does not in any way teach or suggest conducting any reduced dimensionality (RD) NMR experiments, let alone a RD 2D HB,CB,(CG,CD),HD NMR experiment. Thus, Yamazaki cannot overcome the above-noted deficiencies of Szyperski.

Therefore, the combination of Szyperski, Fernández or Gehring, and Yamazaki would not teach the claimed invention. In particular, Szyperski, Fernández, Gehring, and Yamazaki, alone or in combination, fail to teach or suggest a method for obtaining assignments of chemical shift values of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  of a protein molecule by “conducting four reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein (1) a first experiment is selected from the group consisting of a RD three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}\underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment, a RD 3D

H<sub>A</sub>,C<sub>A</sub>(CO),N,HN NMR experiment, and a RD 3D H,C(C-TOCSY-CO),N,HN NMR experiment for obtaining sequential correlations of chemical shift values; (2) a second experiment is selected from the group consisting of a RD 3D HNNCAHA NMR experiment, a RD 3D H<sup>α/β</sup>,C<sup>α/β</sup>,N,HN NMR experiment, and a RD 3D HNN<CO,CA> NMR experiment for obtaining intraresidue correlations of chemical shift values; (3) a third experiment is a RD 3D H,C,C,H-COSY NMR experiment for obtaining assignments of sidechain chemical shift values; and (4) a fourth experiment is a RD two-dimensional (2D) HB,CB(CG,CD),HD NMR experiment for obtaining assignments of aromatic sidechain chemical shift values (emphasis added)” as set forth in claims 91-102, 131, and 132.

In view of the failure of the combination of Szyperski, Fernández, Gehring, and Yamazaki to establish even a *prima facie* case of obviousness, the obviousness rejection based on these references is improper and should be withdrawn.

The rejection of claims 103-130 under 35 U.S.C. § 103(a) for obviousness over Szyperski in view of Fernández or Gehring and Yamazaki, as applied to claims 91-102 and 131-132 above, and further in view of Schirra, “Three Dimensional NMR Spectroscopy” <http://www.cryst.bbk.ac.uk/PPS2/projects/schirra/html/3dnmr.htm> (1996) (“Schirra”) or “Cell Cycle/Gene Regulation,” <http://daisy.bio.nagoya-u.ac.jp/golab/pdb/pdb2nmb.txt> (1998) (“Cell Cycle Protocol”) is respectfully traversed.

Szyperski, Fernández, Gehring, and Yamazaki are described *supra*. Additionally, neither Szyperski, Fernández, Gehring, nor Yamazaki teaches or suggests conducting a RD 3D H,C(C-TOCSY-CO),N,HN NMR experiment, as claimed.

Schirra and Cell Cycle Protocol are cited for teaching various NMR experiments for assigning chemical shifts of various nuclei in the backbone and side chains of proteins via different pathways, the combination of which leads to the complete assignment of NMR spectra. However, both Schirra and Cell Cycle Protocol merely disclose conventional NMR experiments and do not in any way teach or suggest conducting any reduced dimensionality (RD) NMR experiments, let alone a RD 3D H,C(C-TOCSY-CO),N,HN NMR experiment. Thus, neither Schirra nor Cell Cycle Protocol can overcome the above-noted deficiencies of Szyperski, Fernández, Gehring, and Yamazaki.

Therefore, the combination of Szyperski, Fernández or Gehring, Yamazaki, Schirra, and Cell Cycle Protocol would not teach the claimed invention. In particular, Szyperski, Fernández, Gehring, Yamazaki, Schirra, and Cell Cycle Protocol, alone or in

combination, fail to teach or suggest a method for obtaining assignments of chemical shift values of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  of a protein molecule by “conducting four reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein (1) a first experiment is selected from the group consisting of a RD three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}\underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment, a RD 3D  $\text{H}_\alpha\text{A},\text{C}_\alpha(\text{CO}),\text{N},\text{HN}$  NMR experiment, and a RD 3D  $\text{H}_\alpha\text{C}_\alpha(\text{C}-\text{TOCSY}-\text{CO}),\text{N},\text{HN}$  NMR experiment for obtaining sequential correlations of chemical shift values; (2) a second experiment is selected from the group consisting of a RD 3D  $\text{HNNCAHA}$  NMR experiment, a RD 3D  $\underline{\text{H}}^{\alpha/\beta},\underline{\text{C}}^{\alpha/\beta},\text{N},\text{HN}$  NMR experiment, and a RD 3D  $\text{HNN}<\text{CO},\text{CA}>$  NMR experiment for obtaining intraresidue correlations of chemical shift values; (3) a third experiment is a RD 3D  $\text{H}_\alpha\text{C}_\alpha,\text{C}_\alpha\text{H}$ -COSY NMR experiment for obtaining assignments of sidechain chemical shift values; and (4) a fourth experiment is a RD two-dimensional (2D)  $\text{H}_\alpha\text{B},\text{C}_\alpha\text{B},(\text{CG},\text{CD}),\text{HD}$  NMR experiment for obtaining assignments of aromatic sidechain chemical shift values (emphasis added)” as set forth in claim 91, from which claims 103-130 depend.

In view of the failure of the combination of Szyperski, Fernández, Gehring, Yamazaki, Schirra, and Cell Cycle Protocol to establish even a *prima facie* case of obviousness, the obviousness rejection based on these references is improper and should be withdrawn.

The objection to the drawings is obviated in view of the corrected formal drawings submitted herewith. The figures in the attached replacement sheets have been enlarged and improved in order to render them more readable. No new matter has been added.

Finally, applicant hereby requests that the examiner consider the February 25, 2002, Supplemental Information Disclosure Statement, indicate such consideration by initialing the accompanying PTO-1449 form, and return the initialed PTO-1449 form with the next communication from the U.S. Patent and Trademark Office.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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